## REMARKS

Claims 1-91 are currently pending in the application. Claims 1-37, 39 and 53-90 are canceled. Claims 38, 40-41, 43, 45-46, 50, and 91are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Formal Matters

*Further Restriction*

The Examiner asserts that claim 39, drawn to a method to monitor activity of an enzyme comprising removal of a phosphate moiety was improperly included in the elected Group III.1. Applicants have accordingly cancelled claim 39 without prejudice.

*Title/Abstract*

The Examiner asserts that the Title and Abstract must be amended as they are not descriptive, and do not reflect the elected invention. Applicants have thus amended the Title and have provided a new abstract to more appropriately reflect the scope of the claimed invention.

Rejection of Claims 38, 40-52, and 91 Under 35 U.S.C. 112, Second Paragraph

The Examiner has rejected claims 38, 40, 41 and 91 under 35 U.S.C. 112, second paragraph as reciting unelected subject matter. Applicants have accordingly amended claims 38, 40, 41, and 91 to reflect the elected invention.

The Examiner has rejected claims 38, 40-52 and 91 for being vague and indefinite in the recitation of "binding partner". The Examiner asserts that the specification is vague as to a standard for ascertaining the requisite composition, and that one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. Applicants respectfully disagree.

The specification defines the term "binding partner" at page 7, teaching that a "binding partner" is "a polypeptide or fragment thereof (a peptide) that binds to (associates with) a polypeptide comprising a coiled-coil according to the invention. A binding partner usually will

contain a coiled-coil and an engineered site , if these are required for binding." Thus, in the context of amended claim 38: an "isolated polypeptide which associates with a binding partner in a coiled-coil dependent manner", the meaning of a "binding partner" is clear. Contrary to the Examiner's assertion that the specification does not provide a "standard for ascertaining the requisite composition", the specification provides a clear standard: a polypeptide is a binding partner if it binds or associates with a polypeptide comprising a coiled-coil of the invention. No further characterization of a binding partner is required for one of skill in the art to understand fully the scope of the claimed invention. Since the purpose of the requirements of section 112, second paragraph is essentially to make sure that the claims serve notice to a potential infringer of the scope of the claimed invention, Applicants submit that one of skill in the art would be able to determine from the language of the claims, coupled with the express definition in the specification, whether they were practicing the claimed invention or not; that is, whether a particular polypeptide in their possession was a binding partner. Applicants accordingly request that the rejection be reconsidered and withdrawn.

The Examiner has also rejected claims 38, 40-52 and 91 as being vague and indefinite for the recitation of the alleged relative phrase "polypeptide comprising a coiled-coil". Applicants respectfully disagree. At the outset, Applicants point out that the present claims do not recite the phrase "polypeptide comprising a coiled-coil" at all, but instead recite "an isolated polypeptide which associates with a binding partner in a coiled-coil dependent manner."

The Examiner asserts that the claim is indefinite because the term "coiled-coil" describes spatial orientation rather than the structure of the polypeptide, and that it is thus not possible to determine what products are embraced within the scope of the claim. Applicants respectfully disagree. The phrase recited in the claims is "an isolated polypeptide which associates with a binding partner in a coiled-coil dependent manner", thus it is clear from the claim that to meet the limitations, the binding of a given isolated polypeptide to its binding partner must depend on the presence of a coiled-coil structure in the isolated polypeptide. Thus, one of skill in the art would readily understand if a given isolated polypeptide would fall under this limitation by determining whether the binding of the isolated polypeptide to a binding partner was coiled-coil dependent. Support for this amendment to claim 38 is found in the specification at p. 11, lines

17-18, wherein it is stated, "[i]t is preferred that the polypeptide associates via the coiled-coil with another coiled-coil containing polypeptide...". Additional support for amended claim 1 is found in the specification at p. 38, lines 20-22, wherein it is stated, "it is essential that sufficient distance be placed between the donor and acceptor by the linker and/or polypeptides comprising coiled-coils to ensure that FRET does not occur unless the two coiled-coils dimerize." Additional support is found in Example 3, which discloses two proteins interacting via a coiled-coil. It is stated at p.73, lines 29-31, "the coiled-coil sequence 2 is lysine-rich and will not homodimerize, but will heterodimerize with coiled-coil 1...".

In the event, however, that the Examiner, after consideration of the pending claims, rejects the phrase "associates with a binding partner in a coiled-coil dependent manner" under the same rationale used to reject the phrase "polypeptide comprising a coiled-coil", Applicants provide the following remarks.

The Examiner asserts, "[c]oiled coil is a relative term describing spatial orientation rather than structure of the polypeptide. Besides the structural requirements, appearance of the coiled coil, spatial orientation depends on a number of environmental factors in the medium." This rejection is the same rejection set out in the Final Office Action mailed on 12/19/00 in parent application serial no. 09/146,54. In that action, the Examiner cited Lupas et al., 1996, (TIBS, 21:375-82) p. 379, last paragraph as support for this assertion.

In their response, Applicants submitted a Rule 1.132 Declaration by Dr. Derek N. Woolfson, a copy of which is attached hereto. It is disclosed in the attached Rule 1.132 declaration of Dr. Derek N. Woolfson that the coiled-coil proteins of the instant application comprise a stable, environmentally inert background structure into which sites for specific chemical modification by enzymes can be inserted. Therefore, the coiled coil proteins of the instant invention are designed specifically to exhibit predictable behavior that is not dependent upon environmental factors.

The Examiner has also stated, "[n]o standard of reference has been provided in the instant disclosure with which to determine whether a particular organ is a 'polypeptide comprising a coiled-coil' or not." Applicants submit that both the instant application and Lupas et al., 1996,

(TIBS, 21:375-82), cited above by the Examiner, disclose two hallmark structures of the coiled-coil.

It is stated at p. 375, column 2 of Lupas et al., "[t]he hallmark of coiled coils is the distinctive packing of amino acid sidechains in the core of the bundle, called knobs-into holes, in which a residue from one helix (knob) packs into a space surrounded by four sidechains of the facing helix (hole), directly to the side of the equivalent residue from the facing helix... " The instant application teaches at p.26, lines 7-10, "[i]n structural terms coiled-coil helical bundles have between 2 and 5 helices which are offset at roughly 20° to adjacent strands with the hydrophobic sidechains interdigitating in the interface between helices in what is termed the "knobs into holes" packing (Crick, 1953, Acta. Crystallogr., 6: 689-697)."

Both Lupas et al. and the instant application also disclose that coiled-coil structures can be adequately described by spatial arrangements of residues in a sequence referred to as the heptad repeat. The instant application teaches at p. 25, line 30- p. 26, line 2, "a sequence hallmark of a predominance of hydrophobic residues (in particular alanine, isoleucine, leucine, methionine or valine) spaced 3 and 4 residues apart in the primary sequence which is repeated three or more times in near or exact succession (canonical heptad coiled-coil repeat, abbreviated to $(3,4)_n$ where n=3 or greater)." Lupas et al. teach at p. 377, box 1, that the heptad repeat arrangement can be used to search for coiled-coil structures in protein sequences.

The specification also teaches in Example 1, methods of determining if a coiled-coil structure exists, e.g., by CD helical signal, concentration dependence of helical signal, and co-operative unfolding curves. Examples 1 and 3 of the instant application disclose methods of detecting a polypeptide and a binding partner associated in a coiled-coil manner.

Thus, in view of the above, Applicants submit that the instant application clearly teaches standards of reference for identifying a polypeptide comprising a coiled-coil, and methods for identifying the claimed polypeptides of claims 38, 40-52, and 91.

The Examiner has also rejected claims 41 and 43 for alleged lack of antecedent basis for the phrases "said mixing step" and "said fluorescence emitting means". Applicants have amended both claims 41 and 43 to include proper antecedent basis.

Applicants submit that all of the substantive rejections under section 112, second paragraph have been addressed and satisfactorily rebutted. Applicants accordingly request that the rejections be reconsidered and withdrawn.

Rejection of Claims 41-52 Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected claims 41-52 under 35 U.S.C. section 112, first paragraph because the specification, while being enenabling for methods wherein a reporter moiety and its binding partner are the same and the binding is dimerization, does not reasonably provide enablement for any other "binding moiety". The Examiner asserts that, except for the particular example where the binding partner is the same as the reporter molecule, and the binding is dimerizaiton (Applicants assume that the Examiner's use of the term "dimerization" refers to the formation of homodimers), alternative meanings of a binding partner are not disclosed, and no working examples are present. Applicants respectfully disagree and traverse.

To support the assertion that the specification is enabling only for dimers wherein the binding partner is the same as the isolated polypeptide, the Examiner points to paragraphs 123, 144, and 171 of the published application. Each of these paragraphs describes an embodiment of the invention in which an isolated polypeptide and binding protein form a dimer. The Examiner has apparently equated the use of the term "dimer" to mean a "homodimer". This interpretation is not supported by either the specification or the state of the art at the time the application was filed. For example, the specification teaches at page 9, lines 19-22 teaches that a "dimer" refers to a "polypeptide...and its binding partner in the associated or bound state" and thus is not limited to the isolated polypeptide and binding partner being the same protein. The specification teaches further on page 28, lines 21-22 that a coiled-coil domain is structurally conserved among many proteins that interact to form homo- or heterodimeric oligomers (the reference to coiled-coils is relevant because the isolated polypeptide of the invention binds to a binding protein in a coiled-coil dependent manner). Thus, the specification, including paragraphs 123, 144, and 171

of the published application teach that the isolated polyepeptide can associate with a binding protein to form a dimer. Dimers are known in the art to include both heterodimers and homodimers (See, e.g., Oxford Dictionary of Biochemistry and Molecular Biology, 2003 Oxford University Press: dimer defined as "any macromolecular structure in which two (either identical or nonidentical) subunits are noncovalently associated"). The specification teaches numerous coiled-coil proteins (page 30-32) which are known in the art to form heterodimers with a binding protein. For example, as of Applicants' filing date, the coiled-coil protein cJUN was known to heterodimerize with more than 30 different binding proteins, and the coiled-coil protein cFOS was known to heterodimerize with 15 different binding proteins (See, e.g., Chinenov and Kerppola (2001) Oncogene 20:2438).

Thus, Applicants submit that the teachings in the specification combined with the general level of knowledge and skill in the art would permit the ordinary skilled artisan to make and use the claimed invention without undue experimentation. The Examiner asserts, to the contrary, that there are no working examples which correspond to binding between an isolated polypeptide and binding protein wherein the two proteins are not the same. Applicants respectfully remind the Examiner that the absence of working examples is not fatal to a finding of enablement. See, e.g., *In re Borkowski*, 422 F.2d 904 (CCPA 1970). The present specification does, in fact, provide several working examples of the invention, albeit illustrating the invention in a homodimeric model. As noted above, however, the teachings of the specification are not limited to homodimers and provide multiple examples of coiled-coil proteins which are capable of forming known heterodimers with myriad binding proteins. Moreover, the specification teaches how one of skill in the art would test a particular heterodimer (or any dimer for proteins) to determine whether it fulfills the requirements of the claimed method. Applicants refer the Examiner to *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-504, 190 USPQ 214, 217-19 (CCPA 1976)), which states:

> The test [for enablement] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

Applicants submit that the specification combined with the knowledge of one of ordinary skill in the art permits the making and using of the claimed invention without undue experimentation. To reject the claims for lack of enablement, the Examiner must make specific findings of fact, supported by the evidence, as to why one of skill in the art would not be able to practice the invention, given the extensive teachings provided by Applicant, without undue experimentation. MPEP 2164.04. In addition, the Examiner is required to provide specific technical reasons why undue experimentation would be required to practice the invention. Id. Applicant submits that, other than stating that undue experimentation would be required to practice the invention, *the Examiner has not pointed out any technical, factual reasons why this would be the case.*

Applicants assert that the claims are enabled commensurate with their scope and request that the rejection be reconsidered and withdrawn.

Rejection of Claims 38, 40, 45, 49-52, and 91 Under 35 U.S.C. 103(a)

The Examiner has rejected claims 38, 40, 45, 49-52 and 91 as being obvious over Tsien et al. (U.S. Patent 6,197,928). The Examiner asserts that Tsien et al. teach a method for determining the concentration of an analyte in a sample comprising contacting the sample with a binding protein having an analyte binding domain, wherein binding of the analyte permits a donor/acceptor FRET pair on the binding protein to come into close proximity so as to emit a signal. The Examiner asserts that modifying the method of Tsien et al to monitor the phosphorylating activity of a kinase would have been obvious to one of skill in the art, "since a kinase is one of the possible binding proteins used in the method." Applicants respectfully disagree with the Examiner.

To establish a *prima facie* case of obviousness, several basic criteria must be met. Those that are most relevant to the present rejection are the requirement that (1) the prior art reference (or references when combined) must teach or suggest *all the claim limitations* (*In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974)), and (2) that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings (*In re Vaeck*,

947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)). Applicants submit that the teachings Tsien et al do not fulfill these requirements.

*Tsien et al do not teach each element of the claimed invention*

Tsien et al. teach a method for detecting the presence of an analyte in a sample by contacting the sample with a fluorescent indicator comprising a binding moiety, and donor and acceptor fluorescent moieties. Upon binding of the analyte by the binding moiety, a conformational change is induced which brings the donor and acceptor in close spatial proximity so as to generate a fluorescent signal. The focus of the invention taught by Tsien et al. is the binding of the analyte to the binding moiety. There is no teaching in Tsien et al. of an isolated polypeptide which associates with a binding partner in a coiled-coil dependent manner as required by the instant claims. Tsien et al. teaches examples of binding moieties which may be used in the invention, including calmodulin, cGMP-dependent protein kinase, steroid hormone receptors, protein kinase C, inositol-1, 4, 5-triphosphate receptor, and recoverin. None of these proteins comprise coiled-coils, and thus none of these proteins bind to a binding partner in a coiled-coil dependent manner. Moreover Tsien et al. does not contain any teaches relating to coiled-coil proteins. Tsien et al. also does not teach an isolated polypeptide comprising a "non-natural site" sufficient for the addition of a phosphate moiety. The Examiner asserts that with respect to a "site sufficient for the addition of a [phosphate] moiety", that every protein contains a plurality of residues suitable for the addition of a phosphate moiety. The Examiner ignores, however, that the instant claims require a "non-natural site" sufficient for the addition of a phosphate moiety; such a site is not taught by Tsien et al. The Examiner also asserts that the term "engineered" refers to the way of preparing the claimed compound rather than a structural distinction. Applicants point out that the instant claims do not recite the term "engineered", and thus assume that the Examiner's remarks do not reflect a consideration of the pending claims.

*There is no motivation to make the suggested modification of Tsien et al.*

Applicants also submit that one of skill in the art would not be motivated to modify the teachings of Tsien et al. to arrive at the presently claimed invention. The present claims require a method for monitoring enzyme activity by monitoring the addition of a phosphate moiety to an

isolated polypeptide which associates with a binding partner in a coiled-coil dependent manner, wherein the association is dependent on the addition of the phosphate moiety. The Examiner makes the assumption that one of skill in the art would have been motivated to modify Tsien et al. to achieve the claimed invention because it would be "obvious to one skilled in the art that, as a kinase is one of the possible binding proteins used in the method, the method can be used to monitor phosphorylating activity of a kinase." Applicants fail to understand the rationale behind the Examiner's assertion. What the present invention accomplishes is a way to monitor enzyme activity by providing a system wherein a detectable signal is generated in response to the phosphorylation dependent binding of a protein to its binding partner. The mere fact that Tsien et al teach that one embodiment of a binding moiety can be a kinase, does not amount to motivation to modify the teachings of Tsien et al. to *monitor the activity of that kinase.* In order to achieve the claimed invention, Tsien et al. would have to provide sufficient teachings to motivate one of skill in the art to select a binding moiety which will bind to its binding partner in a coiled-coil dependent manner; to introduce into the binding moiety a non-natural site for the addition of a phosphate moiety, and then to use the modified binding moiety in a method to monitor the activity of an enzyme wherein the binding of the binding moiety to its binding partner (the analyte in Tsien et al) is dependent on the addition of a phosphate to the binding moiety. As noted above, Tsien et al. is silent as to coiled-coil proteins, is silent as to modification of such a protein to include a non-natural site sufficient for the addition of a phosphate moiety.

Applicants accordingly submit that not only is there no motivation in Tsien et al. to modify the teachings therein to arrive at the claimed invention, but that even if one of skill in the art were to try to use the proteins taught by Tsien et al to monitor enzyme activity, the modified method would still not include all of the expressly recited elements of the claimed invention. Therefore, the present claims are not obvious in view of Tsien et al. and Applicants request that the rejection be reconsidered and with drawn.

Rejection of Claims 38, 40, 49, and 91 Under 35 U.S.C. §102(b)

The Examiner has rejected claims 38, 40, 49, and 91 under 35 U.S.C. §102(b) as being anticipated by Bastaenis et al. The Examiner asserts that Bastaenis et al. teach monitoring the activity of protein kinase C by "monitoring the interaction between labeled kinase (and hence monitoring of the addition of phosphate moiety) and fluorescently labeled antibody." Applicants respectfully disagree.

At the outset, Applicants respectfully remind the Examiner that a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. See, e.g., *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628 (Fed. Cir. 1987). In this case, Bastaenis et al do not teach several elements of the subject claims. Bastaenis et al. does not teach an isolated protein which associates with a binding partner in a coiled-coil dependent manner. Bastaenis et al. also does not teach that the isolated polypeptide comprises a non-natural site sufficient for the addition of a phosphate moiety. Bastaenis et al. does not teach a method of monitoring the activity of an enzyme wherein the binding of the isolated polypeptide to its binding partner is dependent on the addition of a phosphate moiety to the non-natural site sufficient for the addition of a phosphate moiety. Furthermore, Bastaenis et al. do not teach the addition of a phosphate as asserted by the Examiner. Bastaenis et al. merely teach binding of an antibody to the catalytic site of a kinase; no phosphorylation is involved. Thus, Bastaenis et al. does not teach each element of the claimed invention, and therefore, cannot anticipate the claims.

Applicants submit that Bastaenis et al. does not even teach what the Examiner asserts that it teaches. Bastaenis et al. teach a method for determining whether PKC is translocated to the cell nucleus in an intact, or fragmented form. To accomplish this, Bastaenis et al. labeled the PKC regulatory domain with Cy3 and labeled an anti-PKC antibody with Cy5 (which antibody binds to the PKC catalytic domain). The Cy3 and Cy5 can act as a FRET pair, and thus detection of a fluorescent signal in the cell nucleus is indicative of the translocation of intact PKC. There is no teaching in Bastaenis et al. relating to monitoring the activity of an enzyme, based on the phosphorylation of a peptide or its binding partner. The Examiner's assertion that monitoring interaction between PKC and a fluorescently labeled antibody is "hence monitoring of the addition of phosphate moiety" is not factually correct.

Taken together, the lack of teaching in Bastaenis et al. of an isolated polypeptide comprising a non-natural site sufficient for the addition of a phosphate moiety, and which binds to a binding partner in a coiled-coil dependent manner, and wherein the binding of the polypeptide to the binding partner is dependent on the addition of a phosphate moiety, coupled with a failure to teach a method of monitoring enzyme activity, establishes clearly that Bastaenis et al. do not teach each element of the claimed invention, and therefore does not anticipate the claimed invention.

Applicants accordingly request that the rejection be reconsidered and withdrawn.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: 11/15/04

Name: Kathleen M. Williams
Registration No.: 34,380
Customer No.: 29933
Palmer & Dodge LLP
111 Huntington Avenue
Boston, MA 02199-7613
Tel. (617) 239-0100